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In re Patent Application of:
Arne Mork et al.

Application No.: Not Yet Assigned

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Art Unit: N/A

For: COMBINATION THERAPY WHEREIN A SEROTONIN REUPTAKE INHIBITOR IS USED

Examiner: Not Yet Assigned

CLAIM FOR PRIORITY AND SUBMISSION OF DOCUMENTS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicant hereby claims priority under 35 U.S.C. 119 based on the following prior foreign application filed in the following foreign country on the date indicated:

Country	Application No.	Date
Denmark	PA200200943	June 20, 2002

A certified copy of the aforesaid Danish Patent Application was received by the International Bureau on August 19, 2003 during the pendency of International Application No. PCT/DK03/00412. A copy of Form PCT/IB/304 is enclosed.

Dated: November 29, 2004

Respectfully submitted,

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Kongeriget Danmark

Patent application No.: PA 2002 00943

Date of filing: 20. June 2002

Applicant:
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Title: The combination of a serotonin reuptake inhibitor and a GABAb receptor antagonist

IPC: -

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.

Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

04 July 2003

Åse Damm

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
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PATENT- OG VAREMÆRKESTYRELSEN

The combination of a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist

The present invention relates to the use of certain compounds, and to compositions of compounds having serotonin reuptake inhibiting activity and $GABA_B$ antagonistic, partial agonistic or inverse agonistic activity for the treatment of depression and other affective disorders. The combined serotonin reuptake inhibiting effect and the $GABA_B$ antagonistic, partial agonistic or inverse agonistic effect may reside within the same chemical entity or in two separate chemical entities.

Background

Selective serotonin reuptake inhibitors (hereinafter referred to as SSRIs) have become first choice therapeutics in the treatment of depression, certain forms of anxiety and social phobias, because they are effective, well tolerated and have a favourable safety profile compared to the classic tricyclic antidepressants.

However, clinical studies on depression and anxiety disorders indicate that non-response to SSRIs is substantial, up to 30%. Another, often neglected, factor in antidepressant treatment is compliance, which has a rather profound effect on the patient's motivation to continue pharmacotherapy.

First of all, there is the delay in therapeutic effect of SSRIs. Sometimes symptoms even worsen during the first weeks of treatment. Secondly, sexual dysfunction is a side effect common to all SSRIs. Without addressing these problems, real progress in the pharmacotherapy of depression and anxiety disorders is not likely to happen.

In order to cope with non-response, psychiatrists sometimes make use of augmentation strategies. Augmentation of antidepressant therapy may be accomplished through the co-administration of mood stabilizers such as lithium carbonate or triiodothyronin or by the use of electroshock.

In 1993, an augmentation strategy with pindolol was described by Artigas et al. in *Trends Pharmacol. Sci.* **1993**, *14*, p 262-263. Artigas' idea is based on intracerebral microdialysis experiments in animals. In fact, later neurochemical studies built on the desensitization hypothesis by Blier and co-workers stated that the delay in therapeutic effect of antidepressants is related to a gradual desensitization of 5-HT autoreceptors (Blier et al. *J. Clin. Psycipharmacol.* **1987**, *7 suppl.* 6, 24S-35S). A key point in their hypothesis is that the effects of SSRIs on the release-controlling somatodendritic autoreceptors (5-HT_{1A}) limit the release of 5-HT in terminal areas and thus the effect of 5-HT uptake inhibition in those regions. This is supported by microdialysis experiments in rats, showing that the increase in extracellular 5-HT elicited by a single dose of an SSRI is augmented by co-administration of a 5-HT_{1A} autoreceptor antagonist (Invernizzi et al. *Brain Res*, **1992**, *584*, p 322-324 and Hjorth, S., J. *Neurochem*, **1993**, *60*, p 776-779).

The effect of combined administration of a compound that inhibits serotonin reuptake and a 5-HT_{1A} receptor antagonist has been evaluated in several studies (Innis, R.B. et al. *Eur. J. Pharmacol.* **1987**, *143*, p. 1095-204 and Gartside, S.E., *Br. J. Pharmacol*, **1995**, *115*, p 1064-1070, Blier, P. et al. *Trends in Pharmacol. Science* **1994**, *15*, 220). In these studies it was found that 5-HT_{1A} receptor antagonists would abolish the initial brake on 5-HT neurotransmission induced by the serotonin reuptake inhibitors and thus produce an immediate boost of 5-HT transmission and a rapid onset of therapeutic action.

Several patent applications have been filed which cover the use of a combination of a 5-HT_{1A} antagonist and a serotonin reuptake inhibitor for the treatment of depression (see e.g. EP-A2-687 472 and EP-A2-714 663).

Another approach to increase terminal 5-HT would be through blockade of the 5-HT_{1B} autoreceptor. Microdialysis experiments in rats have indeed shown that increase of hippocampal 5-HT by citalopram is potentiated by GMC 2-29, an experimental 5-HT_{1B} receptor antagonist.

Several patent applications covering the combination of an SSRI and a 5-HT_{1B} antagonist or partial agonist have also been filed (WO 97/28141, WO 96/03400, EP-A-701819 and WO 99/13877).

It has now surprisingly been found that a $GABA_B$ antagonist will augment the effect of an SSRI on extracellular 5-HT levels

γ-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the brain; up to 50% of all central synapses are GABA-ergic (Paredes R.G. & Agmo A., Neuroscience and Biobehavioural Reviews, vol 16: pp 145-170 (1992)).

Noradrenaline, dopamine and serotonin (5-HT) are all under inhibitory control of GABA (Haefely W. The role of GABA in anxiolytic/antidepressant drug action. Elliott M.M., Heal D.J. & Marsden C.A. (eds), pp 151-168, John Wiley & Sons, New York (1992)). There are two subtypes of GABA receptors, $GABA_A$ and $GABA_B$, which have been extensively studied and their influence on 5HT nerve function and release have been performed.

Thus stimulation of the $GABA_A$ receptor with the agonist muscimol reduced 5-HT cell activity & 5-HT release in the raphé nuclei (Tao R. & Auerbach S.B. J. Psychopharmacology vol 14(2): pp 100-113 (2000)) and blockade of $GABA_A$ receptors increases firing and subsequently elevates levels of extracellular 5-HT (see below - bicuculline & Tao R. *et al.*, British Journal of Pharmacology vol 119: pp 1375-1384 (1996)).

The $GABA_B$ agonist baclofen produced an increase in 5-HT in the raphé and reduction in the forebrain (Tao R. *et al.*, British Journal of Pharmacology vol 119: pp 1375-1384 (1996)) and when administered by itself the $GABA_B$ antagonist, phaclofen, is devoid of effect on 5-HT levels in either the raphé (Abellán M.T. *et al.*, Neuroreport vol 11: pp941-945 (2000); Tao R. *et al.*, British Journal of Pharmacology vol 119: pp 1375-1384 (1996)) or in the forebrain (see below & Tao R. *et al.*, British Journal of Pharmacology vol 119: pp 1375-1384 (1996)). However, phaclofen, administered either centrally into the hippocampus or systemically was shown to

significally increase the effects of citalopram on extracellular 5-HT levels, as demonstrated in the findings reported here.

It is therefore suggested that the combination of an SSRI and a $GABA_B$ antagonist or a molecule, which has both 5-HT reuptake inhibitory and $GABA_B$ antagonistic properties, would have a better efficacy and faster onset than an SSRI alone.

Antagonism at any $GABA_B$ splice variants, including but not limited to $GABA_{BR1a}$ and $GABA_{BR1b}$ is claimed.

This invention covers both SSRI + $GABA_B$ antagonist in separate or the same molecule.

The invention

The present invention thus provides:

The use of a $GABA_B$ receptor antagonist, inverse agonist or partial agonist for the preparation of a pharmaceutical composition to be used in combination with a serotonin reuptake inhibitor inhibitor.

In particular, the present invention relates to the use of a $GABA_B$ receptor antagonist, inverse agonist or partial agonist for the preparation of a pharmaceutical composition useful for augmenting and/or providing faster onset of the therapeutic effect of a serotonin reuptake inhibitor.

In a preferred embodiment, the invention relates to the use as above wherein the serotonin reuptake inhibitor is used for the treatment of depression, anxiety disorders and other affective disorders, including generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder or social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control

disorders, attention deficit hyperactivity disorder, drug abuse and any other disorder responsive to a SRI.

In another embodiment, the invention relates to the use of

a) a compound which is a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist, or
b) a combination of a compound, which is a serotonin reuptake inhibitor, and a compound, which is a $GABA_B$ receptor antagonist, inverse agonist or partial agonist,

for the preparation of a pharmaceutical composition or kit useful for the treatment of depression, anxiety disorders and other affective disorders, such as generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder and social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to serotonin reuptake inhibitors.

In a further embodiment, the invention relates to a pharmaceutical composition or kit comprising:

a) a compound, which is a serotonin reuptake inhibitor, and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist, or
b) a combination of a compound, which is a serotonin reuptake inhibitor, and another compound, which is a $GABA_B$ receptor antagonist, inverse agonist or partial agonist,

and optionally pharmaceutically acceptable carriers or diluents.

In a yet another embodiment, the invention relates to a method for the treatment of depression, anxiety disorders and other affective disorders, such as generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder,

post traumatic stress disorder and social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to serotonin reuptake inhibitors comprising administering to a person in need thereof a therapeutically effective amount of

a) a compound, which is a serotonin reuptake inhibitor, and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist, or
b) a combination of a compound, which is a serotonin reuptake inhibitor and a compound, which is a $GABA_B$ receptor antagonist, inverse agonist or partial agonist.

In a particular embodiment, a selective serotonin reuptake inhibitor is used according to the invention.

In another particular embodiment, a compound which is selective for the $GABA_B$ receptor is used according to the invention.

In a further embodiment, a compound which is an antagonist, an inverse agonist at the $GABA_B$ receptor is used according to the invention.

The pharmaceutical composition or kit according to the invention may be adapted for simultaneous administration of the active ingredients, or it may be adapted for sequential administration of the active ingredients.

When the pharmaceutical composition is adapted for simultaneous administration, the active ingredients may be contained in the same unit dosage form.

When the pharmaceutical composition or kit is adapted for sequential administration, the active ingredients are contained in discrete dosage forms, optionally contained in the same container or package.

In particular, the present invention relates to the use of, and to pharmaceutical compositions or kits comprising the following combinations:

CGP 55845 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, vilazodone, duloxetine, nefazodone, imipramin, femoxetine and clomipramine,

CGP 62349 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 71982 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 76290 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 76291 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 35348 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 36742 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 46381 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 52432 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

CGP 546926 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imiparmin, femoxetine and clomipramine,

SCH 50911 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

Phaclofen and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

Saclofen and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

2-hydroxysaclofen and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine,

GAS 360 and an SRI selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine .

In a final embodiment, the present invention relates to a method for the identification of compounds useful for the treatment of depression, anxiety disorders and other affective disorders, such as generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder and social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to serotonin reuptake inhibitors, comprising, in any order:

(a) measuring the ability of test compounds to inhibit serotonin reuptake and selecting the compounds that have an IC_{50} value below 20 nM;

(b) measuring the affinity of test compounds to the $GABA_B$ receptor and selecting the compounds.

and thereafter measuring the efficacy of the selected compounds at the $GABA_B$ receptor and selecting the compounds which are antagonists, inverse agonists at the receptor.

Preferred $GABA_B$ ligands show affinity of below 500 nM. Even more preferred are compounds with affinity below 100 nM.

Examples of assays for the selection / detection of $GABA_B$ antagonists, inverse agonists or partial agonists are for example the following:

Binding assay for the detection of compounds with affinity for $GABA_B$ receptors are described in Karla et. al., J. Med. Chem. 1999, 42(11), 2053-2059; or Frydenvang et. al., Chirality, 1994; 6(7); 583-589;

Efficacy assay for the detection of antagonists, partial agonists or inverse agonists at the $GABA_B$ receptors are for example: Kamatchi et. al., Brain Res. , 1990, 506(2), 181-186; or Brauner-Osborne et. al., Br. J. Pharmacol. 1999, 128(7), 1370-1374.

The invention also covers compounds identified according to this method, but is not limited to theses assay methods.

According to the invention, it has been found that co-administration of $GABA_B$ receptor antagonist or inverse agonist and a serotonin reuptake inhibitor produces a significant increase in the level of serotonin in terminal areas, as measured in microdialysis experiments, compared to the administration of the serotonin reuptake inhibitor alone.

According to the invention, animal studies have shown that $GABA_B$ receptor antagonist or inverse agonist may provide fast onset of therapeutic effect of serotonin reuptake inhibitors and potentiate the anxiolytic potential of serotonin reuptake inhibitors.

The use of a combination of $GABA_B$ receptor antagonist, inverse agonist or partial agonist and a serotonin reuptake inhibitor may greatly reduce the amount of serotonin reuptake inhibitor necessary to treat depression and other affective disorders and may thus reduce the side effects caused by the serotonin reuptake inhibitor. In particular, the combination of a reduced amount of SRI and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist may reduce the risk of SSRI-induced sexual dysfunction and sleep disturbances.

Co-administration of a $GABA_B$ receptor antagonist, inverse agonist or partial agonist and a serotonin reuptake inhibitor may also be useful for the treatment of refractory depression, i.e. depression, which cannot be treated appropriately by administration of a serotonin reuptake inhibitor alone. Typically, $GABA_B$ receptor antagonist, inverse agonist or partial agonist may be used as add-on therapy for the augmentation of the response to SRIs in patients where at least 40-60% reduction in symptoms has not been achieved during the first 6 weeks of treatment with an SRI.

Compounds which are both serotonin reuptake inhibitors and $GABA_B$ receptor antagonists, inverse agonists or partial agonists may have the same pharmacological advantages as the combination of a serotonin reuptake inhibitor and a $GABA_B$ receptor

antagonists, inverse agonists or partial agonists, with respect to reduction of side effects, fast onset and in the treatment of treatment resistant patients.

Many antidepressants with serotonin reuptake inhibiting effect have been described in the literature. Any pharmacologically active compound, which primarily or partly exert its therapeutic effect via inhibition of serotonin reuptake in the CNS, may benefit from augmentation with a $GABA_B$ receptor antagonist, inverse agonist or partial agonist.

The following list contains a number of serotonin reuptake inhibitors, which may benefit from augmentation with a $GABA_B$ receptor antagonist, inverse agonist or partial agonist: citalopram, escitalopram, fluoxetine, R-fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, desmethylvenlafaxine, duloxetine, dapoxetine, vilazodone, nefazodone, imipramine, imipramine N-oxide, desipramine, pirandamine, dazepinil, nefopam, befuraline, fezolamine, femoxetine, clomipramine, cianoimipramine, litoxetine, cericlamine, seproxetine, WY 27587, WY 27866, imeldine, ifoxetine, indeloxazine, tiflucarbine, viqualine, milnacipran, bazinaprine, YM 922, S 33005, F 98214-TA, FI 4503, A 80426, EMD 86006, NS 2389, S33005, OPC 14523, alaproclate, cyanodothepine, trimipramine, quinupramine, dothiepin, amoxapine, nitroxazepine, McN 5652, McN 5707, VN 2222, L 792339, roxindole, YM 35992, Ol 77, Org 6582, Org 6997, Org 6906, amitriptyline, amitriptyline N-oxide, nortriptyline, CL 255.663, pirlindole, indatraline, LY 280253, LY 285974, LY 113.821, LY 214.281, CGP 6085 A, RU 25.591, napamezole, diclofensine, trazodone, BMY 42.569, NS 2389, sercloremine, nitroquipazine, ademethionine, sibutramine, desmethylsubitramine, didesmethylsubitramine, clovoxamine vilnazodone. The compounds mentioned above may be used in the form of the base or a pharmaceutically acceptable acid addition salt thereof.

Other therapeutic compounds, which may benefit from augmentation with $GABA_B$ receptor antagonists, inverse agonist or partial agonists, include compounds, which cause an elevation in the extracellular level of 5-HT in the synaptic cleft, although they are not serotonin reuptake inhibitors. One such compound is tianeptine

Accordingly, other compounds than SRIs which cause an elevation in the extracellular level of serotonin, may be used instead of SRIs in every aspect of the invention as described herein.

The above list of serotonin reuptake inhibitors and other compounds, which cause an increase in the extracellular level of serotonin, may not be construed as limiting.

SRIs, which are particularly preferred according to the present invention, include citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine.

The term selective serotonin reuptake inhibitor (SSRI) means an inhibitor of the monoamine transporters which has stronger inhibitory effect at the serotonin transporter than the dopamine and the noradrenaline transporters. Particularly preferred SSRIs according to the invention are citalopram, escitalopram, fluoxetine, fluvoxamine, sertraline, duloxetine, vilnazodone and paroxetine.

The following list contains a number of $GABA_B$ antagonists, partial agonists or inverse agonists, which may be used according to the invention:
CGP-71982, CGP-76290, CGP-76291, CGP-35348, CGP-36742, CGP-46381, CGP-52432, CGP-54626,CGP-55845,CGP-55845, CGP-62349, SCH 50911, GAS-360, Phaclofen, Saclofen, 2-hydroxysaclofen.

In a preferred embodiment, a $GABA_B$ receptor ligand selected from CGP 71982, CGP 76290, CGP 55845 and CGP 62349.

Materials and Methods

Animals

Male albino rats of a Wistar-derived strain (285-320 g; Harlan, Zeist, The Netherlands) were used for the experiments. Upon surgery, rats were housed

individually in plastic cages (35 x 35 x 40 cm), and had free access to food and water. Animals were kept on a 12 h light schedule (light on 7:00 a.m.). The experiments are concordant with the declarations of Helsinki and were approved by the animal care committee of the faculty of mathematics and natural science of the University of Groningen .

Drugs

The following drugs were used: Citalopram hydrobromide (Lundbeck A/S, Copenhagen, Denmark), Phaclofen and (+)-Bicuculline (Sigma, St Louis, USA).

Surgery

Microdialysis of brain serotonin levels was performed using home made I-shaped probes, made of polyacrylonitrile / sodium methyl sulfonate copolymer dialysis fiber (i.d. 220 µm, o.d. 0.31 µm, AN 69, Hospal, Italy). Preceding surgery rats were anaesthetised using isoflurane (O_2/N_2O; 300/300ml/min). Lidocaine-HCl, 10 % (m/v) was used for local anaesthesia. Rats were placed in a stereotaxic frame (Kopf, USA), and probes were inserted into Ventral Hippcampus (V. Hippo, L +4.8 mm, IA: +3.7 mm, V: -8.0 mm) (Paxinos and Watson, 1982). After insertion, probes were secured with dental cement.

Microdialysis experiments

Rats were allowed to recover for at least 24 h. Probes were perfused with artificial cerebrospinal fluid containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$, at a flow-rate of 1.5 µl / min (Harvard apparatus, South Natick, Ma., USA). 15 minute microdialysis samples were collected in HPLC vials containing 7.5 µl 0.02 M acetic acid for serotonin analysis.

Serotonin analysis:

Twenty-µl microdialysate samples were injected via an autoinjector (CMA/200 refrigerated microsampler, CMA, Sweden) onto a 100 x 2.0 mm C18 Hypersil 3 µm

column (Bester, Amstelveen, the Netherlands) and separated with a mobile phase consisting of 5 g/L di-ammoniumsulfate, 500 mg/L EDTA, 50 mg/L heptane sulphonic acid, 4 % methanol v/v, and 30 µl/L of triethylamine, pH 4.65 at a flow of 0.4 ml/min (Shimadzu LC-10 AD). 5-HT was detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, The Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal to noise ratio 3).

Data presentation and statistics

Four consecutive microdialysis samples with less then 20 % variation were taken as control and set at 100 %. Data are presented as percentages of control level (mean ± S.E.M.) in time. Statistical analysis was performed using Sigmastat for Windows (SPSS, Jandel Corporation). Treatments were compared versus controls using two way analysis of variance (ANOVA) for repeated measurements, followed by Student Newman Keuls test. Level of significance level was set at $p<0.05$.

Results

Local administration of GABAa antagonist bicuculline, followed by systemic administration of citalopram (fig 1).

Local administration of 50 µM bicuculline in ventral hippocampus increased serotonin levels by about 150 % (treatment vs. time; $F(1,79) = 5.20$, $P=0.0003$). Post-hoc analysis revealed significance from t=45 to 90 min.

The increase established by systemic administration of 10 µmol/kg s.c. citalopram was not affected by local application of bicuculline (treatment; $F(1,10) = 4.64$, $P= 0.0567$).

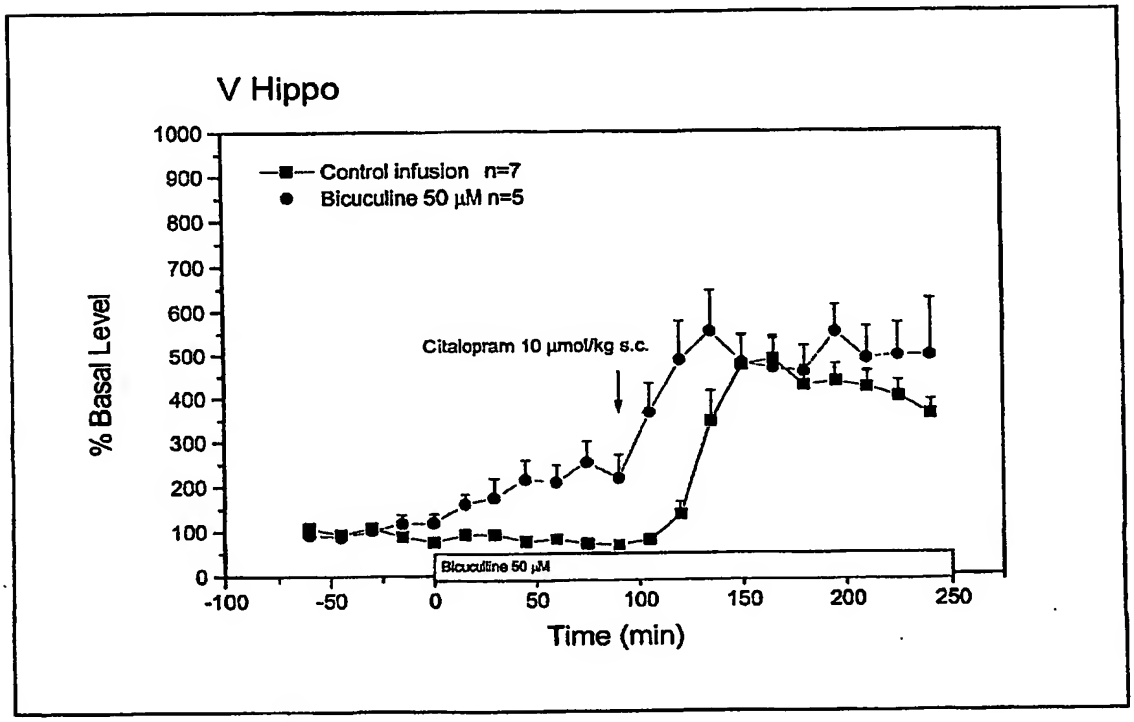

Fig 1. Effect of local administration of bicuculline (50 µM), followed by systemic administration of citalopram 10 µmol/kg s.c.

Local administration of GABAb antagonist phaclofen, followed by systemic administration of citalopram (fig 2).

Local infusion of GABAb antagonist Phaclofen did not have any effect on basal levels of 5-HT in ventral hippocampus ($F(1,9)= 1.44$ $P=0.26$). Systemic administration of citalopram during local administration of phaclofen induced augmented levels of 5-HT (Treatment $F(1,9)=12.21$ $P= 0.0068$, Treatment vs. Time $F(1,112) = 5.03$ $P<0.0001$). Significant differences during post-hoc analysis was attained from t = 75 to 150 min.

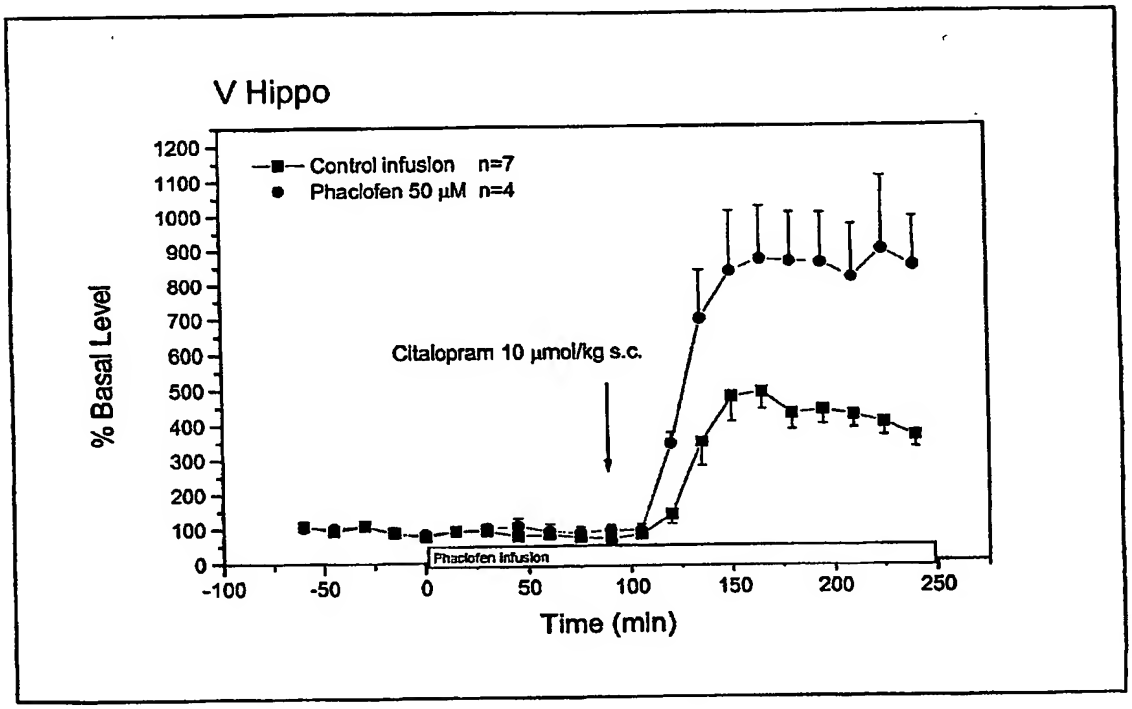

Fig 2. Effect of local administration of phaclofen (50 µM), followed by systemic administration of citalopram 10 µmol/kg s.c.

Simultaneous administration of phaclofen 2 mg/kg s.c. with citalopram 10 µmol/kg s.c. (fig 3).

Co-administration of phaclofen 2 mg/kg s.c with citalopram 10 µmol/kg s.c. elicited enhanced levels of 5-HT when compared to citalopram treatment alone (Treatment $F(1,7)=8.64$ $P=0.021$, Treatment vs. Time, $F(1,98)=6.38$ $P<0.0001$). Post-hoc analysis showed different effects from t=75 to t=135.

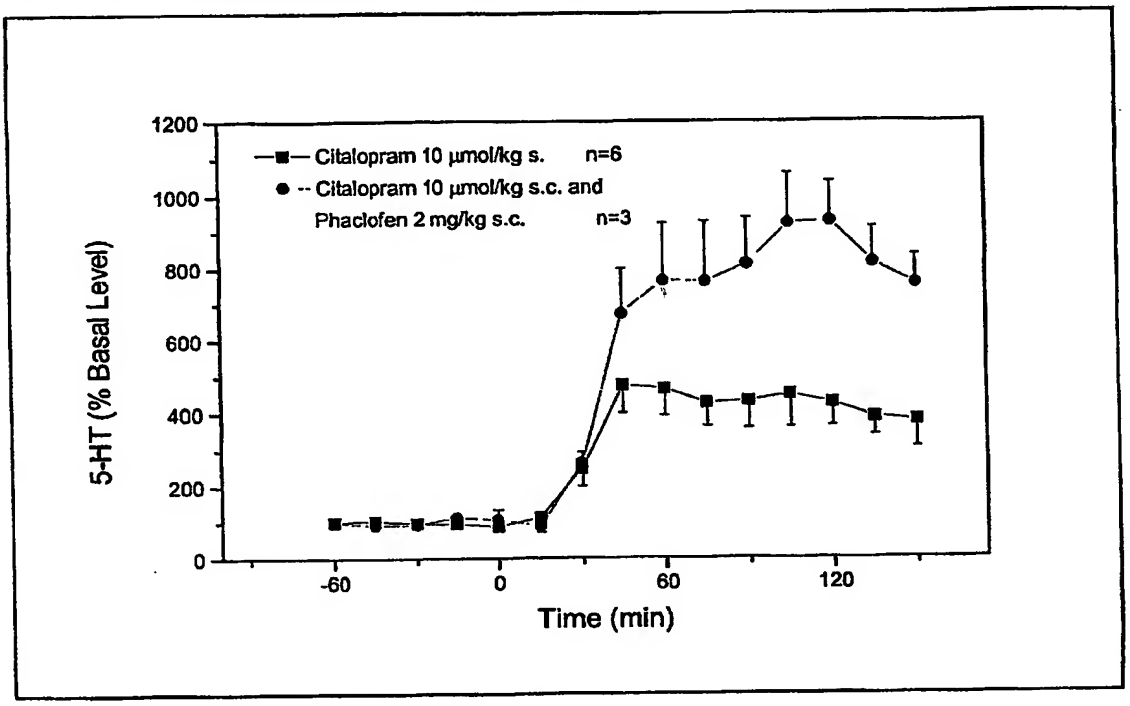

Fig 3. Effect of co-administration of phaclofen (2 mg/kg s.c.), followed by systemic administration of citalopram (10 µmol/kg s.c.).

Claims:

1. The use of a $GABA_B$ receptor antagonist, inverse agonist or partial agonist for the preparation of a pharmaceutical composition to be used in combination with a serotonin reuptake inhibitor inhibitor.

2. The use of a $GABA_B$ receptor antagonist, inverse agonist or partial agonist for the preparation of a pharmaceutical composition useful for augmenting and/or providing faster onset of the therapeutic effect of a serotonin reuptake inhibitor.

3. The use according to claims 1 or 2 wherein the serotonin reuptake inhibitor is used for the treatment of depression, anxiety disorders and other affective disorders, including generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder or social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to a SRI.

4. The use of

a) a compound, which is a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist, or
b) a combination of a compound, which is a serotonin reuptake inhibitor, and a compound, which is a $GABA_B$ receptor antagonist, inverse agonist or partial agonist,

for the preparation of a pharmaceutical composition useful for the treatment of depression, anxiety disorders and other affective disorders, such as generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder and social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to serotonin reuptake inhibitors.

5. The use according to claim 4 wherein a compound, which is a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist, is used for the preparation of the pharmaceutical composition.

6. The use according to claim 4 wherein a combination of a compound, which is a serotonin reuptake inhibitor, and another compound, which is a $GABA_B$ receptor ligand, is used.

7. The use according to claims 1-6 wherein a selective serotonin reuptake inhibitor is used.

8. The use according to claims 1-6 wherein a compound, which is selective for the $GABA_B$ receptor, is used.

9. The use according to claims 1-6 wherein an antagonist or an inverse agonist at the $GABA_B$ receptor is used.

10. The use according to claims 1- 4 and 6-9 wherein the SRI is elected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine.

11. The use according to claims 1-4 and 6-9 wherein the $GABA_B$ receptor ligand is selected from CGP-71982, CGP-76290, CGP-76291, CGP-35348, CGP-36742, CGP-46381, CGP-52432, CGP-54626, CGP-55845, CGP-62349, SCH 50911, GAS-360, Phaclofen, Saclofen, 2-hydroxysaclofen.

12. A pharmaceutical composition comprising:

a) a compound which is a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist or,

b) a combination of a compound, which is a serotonin reuptake inhibitor, and another compound, which is a $GABA_B$ receptor antagonist, inverse agonist or partial agonist,

and optionally pharmaceutically acceptable carriers or diluents.

13. The pharmaceutical composition according to claim 12 comprising a compound, which is a serotonin reuptake inhibitor and a $GABA_B$ receptor antagonist, inverse agonist or partial agonist.

14. The pharmaceutical composition according to claim 12 comprising a combination of a compound, which is a serotonin reuptake inhibitor, and another compound, which is a $GABA_B$ antagonist, inverse agonist or partial agonist.

15. A pharmaceutical composition according to claims 12-14 wherein the serotonin reuptake inhibitor used is a selective serotonin reuptake inhibitor.

16. A pharmaceutical composition according to claims 12-14 wherein the $GABA_B$ antagonist, inverse agonist of partial agonist is selective for the $GABA_B$ receptor.

17. A pharmaceutical composition according to claims 12-14 wherein the $GABA_B$ ligand is a compound, which is an antagonist or an inverse agonist at the $GABA_B$ receptor.

18. A pharmaceutical composition according to claim 14 characterized in that the serotonin uptake inhibitor is selected from citalopram, escitalopram, fluoxetine, sertraline, paroxetine, fluvoxamine, venlafaxine, dapoxetine, duloxetine, vilazodone, nefazodone, imipramin, femoxetine and clomipramine.

19. A pharmaceutical composition according to claim 14 characterized in that the $GABA_B$ ligand is selected from CGP-71982, CGP-76290, CGP-76291, CGP-35348, CGP-36742, CGP-46381, CGP-52432, CGP-54626,CGP-55845,CGP-55845, CGP-62349, SCH 50911, GAS-360, Phaclofen, Saclofen or 2-hydroxysaclofen.

20. A pharmaceutical composition according to claim 12 wherein the pharmaceutical composition is adapted for simultaneous administration of the active ingredients.

21. The pharmaceutical composition according to claim 20 wherein the active ingredients are contained in the same unit dosage form.

22. A pharmaceutical composition according to claim 12 wherein the pharmaceutical composition is adapted for sequential administration of the active ingredients.

23. The pharmaceutical composition according to claims 20 or 22 wherein the active ingredients are contained in discrete dosage forms.

24. A method for the identification of compounds useful for the treatment of depression, anxiety disorders and other affective disorders, such as generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, acute stress disorder, post traumatic stress disorder and social anxiety disorder, eating disorders such as bulimia, anorexia and obesity, phobias, dysthymia, premenstrual syndrome, cognitive disorders, impulse control disorders, attention deficit hyperactivity disorder, drug abuse or any other disorder responsive to serotonin reuptake inhibitors, comprising, in any order:

(a) measuring the ability of test compounds to inhibit serotonin reuptake and selecting the compounds that have an IC_{50} value below 20 nM;

(b) measuring the affinity of test compounds to the $GABA_B$ receptor and selecting the compounds,

and thereafter measuring the efficacy of the selected compounds at the $GABA_B$ receptor and selecting the compounds which are antagonists, inverse agonists or partial agonists at the receptor.

25. A method according to claim 24 wherein the compound has an affinity in step (b) of less than 500 nM;

26. A method according to claims 24 or 25, wherein the compound has an affinity in step (b) of less than 100 nM:

27. A compound identified according to any of the claims 24-27.